

Original Article

Pregnancy-induced metabolic reprogramming in skeletal muscle: a multi-omics interrogation of transcriptional and metabolic adaptations

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Abstract

Pregnancy induces profound physiological adaptations to meet the dynamic nutritional demands of fetal development, including a deliberate reduction in maternal insulin sensitivity to ensure fetal glucose availability. However, excessive insulin resistance may precipitate gestational diabetes mellitus (GDM), increasing the risk of both obstetric complications and long-term metabolic disorders in mothers and offspring. Although the role of adipose tissue in pregnancy-associated metabolic adaptation has been extensively studied, the contribution of skeletal muscle remains poorly understood. Here, we systematically characterize pregnancy-induced molecular and metabolic changes in maternal skeletal muscle through multi-omics profiling. We use transcriptomic, metabolomic, computational single-cell deconvolution, and qPCR validation in an established C57BL/6J mouse pregnancy model (8-week-old females). Pregnancy triggers remarkable skeletal muscle remodelling, featuring histological reorganization with myofiber depletion and expanded endothelial compartments. Concurrent metabolic disturbances include insulin resistance, dysregulated TCA cycle activity, and impaired ubiquinone biosynthesis. This study represents a multi-omics-based systematic elucidation of pregnancy-induced maternal skeletal muscle adaptations. Our findings demonstrate that pregnancy induces profound structural reorganization and metabolic reprogramming in maternal skeletal muscle, characterized by prioritized fetal nutrient provision at the expense of maternal tissue utilization. These observations not only reveal previously unrecognized mechanisms of pregnancy-specific metabolic regulation but also, more importantly, establish a critical theoretical foundation for developing skeletal muscle-targeted intervention strategies to prevent gestational diabetes mellitus.

Key words pregnancy, metabolic adaptation, skeletal muscle, transcriptomic, metabolomics

Introduction

Gestational diabetes mellitus (GDM) has emerged as a major global health challenge, with prevalence rates increasing to 2%–25% across populations worldwide [1]. Defined by the new onset of glucose intolerance, GDM encompasses conditions ranging from newly detected hyperglycemia during pregnancy to pre-existing

impaired glucose tolerance or overt type 2 diabetes mellitus (T2DM). This condition has profound clinical implications: longitudinal studies have revealed that nearly 50% of GDM women develop T2DM within five years post-partum, whereas their offspring face significantly increased risks of obesity and metabolic syndrome [2,3]. This transgenerational metabolic programming

underscores GDM's critical role in the developmental origins of metabolic disorders, highlighting the urgent need to decipher its underlying mechanisms.

The metabolic adaptations of pregnancy, while physiologically necessary to support fetal growth, create a precarious balance that may predispose individuals to GDM. The current understanding emphasizes adipose tissue expansion as the primary driver of pregnancy-induced insulin resistance, accounting for approximately one-third of the recommended gestational weight gain [4–7]. However, emerging evidence indicates that among insulin-responsive tissues, skeletal muscle—more prominently than hepatic tissue—undergoes pregnancy-specific suppression of insulin signaling pathways, in addition to adipose tissue involvement [8]. These findings position skeletal muscle as a potentially crucial mediator of gestational metabolic reprogramming, suggesting that its maladaptation may be instrumental in GDM pathogenesis.

The parallels between GDM and T2DM pathophysiology further reinforce the likely contribution of skeletal muscle [9]. As the tissue responsible for the majority of insulin-stimulated glucose disposal, skeletal muscle is recognized as the primary site of insulin resistance in T2DM patients. Emerging data indicate similar centrality in GDM, where pregnancy-induced skeletal muscle metabolic derangements strongly correlate significantly positively with insulin resistance severity and independently predict GDM risk. This association may be mediated through impaired insulin signal transduction in skeletal muscle [10], mitochondrial dysfunction-induced metabolic inflexibility [11,12], and myokine-mediated systemic inflammation [13,14]. Despite these advances, the molecular mechanisms underlying pregnancy-specific skeletal muscle metabolic remodelling and its causal relationship with GDM require further investigation.

Our approach enables: (1) comprehensive profiling of gene expression dynamics at tissue and single-cell resolutions, (2) identification of dysregulated metabolic pathways, and (3) validation of candidate regulators through qRT-PCR. This study employs an integrated multi-omics framework combining bulk and single-cell transcriptomics with targeted metabolomics to systematically map pregnancy-induced molecular reprogramming in skeletal muscle. Our approach enables comprehensive profiling of gene expression dynamics at tissue and single-cell resolutions and identification of dysregulated metabolic pathways in maternal skeletal muscle. By elucidating the molecular architecture of skeletal muscle adaptation during pregnancy, this study provides critical insights into GDM pathogenesis while revealing novel targets for early detection and intervention.

Materials and Methods

Animal care and experimental design

All animal experimental procedures were approved by the Animal Experiment Center Ethics Committee of Zhejiang University (approval No. ZJU20240390). Female C57BL/6J mice (8–10 weeks old, virgin) from GemPharmatech (Changzhou, China) were housed under standard conditions ($22 \pm 0.5^\circ\text{C}$, 12-h light/12-h dark cycle, 40%–70% humidity) with *ad libitum* access to food and water. For mating, virgin female mice were co-housed with adult male mice at a 1:2 (male:female) ratio. Vaginal plugs were examined every morning, and the presence of a vaginal plug was designated as embryonic day 0.5 (E0.5). Pregnant mice at E17.5 were designated the Preg group ($n = 7$), whereas age-matched non-

pregnant female mice served as the Non-Preg group ($n = 10$). Female virgin C57BL/6J mice (6 weeks old, initial body weight of approximately 12 g) were randomly assigned to either a high-fat diet (HFD; #D12492, Research Diets, New Brunswick, USA; 60% fat, 20% protein) or a normal chow diet (NCD; #D12450B, Research Diets; 10% fat, 20% protein) for two weeks prior to mating. The respective dietary regimens were maintained throughout the subsequent pregnancy period, thereby establishing the NCD ($n = 7$) and HFD ($n = 9$) experimental groups. For mating, 10-week-old male C57BL/6J mice maintained on a standard NCD were used.

Hematoxylin and eosin (H&E) staining

The gastrocnemius muscle samples were fixed in 10% neutral buffered formalin for 24 h, followed by dehydration, paraffin embedding, and sectioning. The tissue sections were stained with hematoxylin for 8 min, rinsed thoroughly, and counterstained with eosin for 3 min. The sections were subsequently dehydrated, cleared, and mounted for histological examination.

In vivo metabolic testing

Unrestrained mice were subjected to fasting for 16 h (for the glucose tolerance test) or 4 h (for the insulin tolerance test), followed by the intraperitoneal administration of glucose (2 g/kg body weight) or insulin (0.8 units/kg body weight), respectively, for the corresponding assessments. Blood glucose levels were measured from tail vein samples at 0, 30, 60, and 120 min using a glucometer.

Biochemical analysis

The mice were fasted overnight and subsequently euthanized by cervical dislocation. Blood samples were collected in EDTA-coated tubes, followed by centrifugation to separate the plasma, which was then stored at -80°C . Serum triglyceride (TG) and high-density lipoprotein (HDL) levels were measured using a TBA-120FR fully automated biochemical analyzer (Toshiba, Tokyo, Japan).

RNA extraction and transcriptome analysis

Total RNA was isolated from the gastrocnemius muscle via an RNA fast200 kit (Feijie, Shanghai, China). RNA purity and integrity were assessed using a NanoDrop ND-1000 spectrophotometer and Agilent 2100 Bioanalyzer (Santa Clara, USA). Samples with an RNA integrity number (RIN) > 7.0 were selected for cDNA synthesis and sequenced on an Illumina HiSeq2500 platform. Differentially expressed genes (DEGs) with $|\log_2 \text{foldchange (FC)}| > 1$ and P value < 0.05 were identified. Gene Ontology (GO) functional annotation and KEGG pathway enrichment analyses were subsequently performed.

Single-cell data deconvolution analysis

CIBERSORTx is a machine learning-based analytical toolkit specifically designed for estimating cell type abundance in bulk RNA sequencing (RNA-seq) and microarray data [15,16]. In this study, we employed the GSE197017 [17] single-cell RNA sequencing (scRNA-seq) dataset to construct a single-cell signature matrix via CIBERSORTx. Following the standardized protocol for formatting and uploading the single-cell reference matrix file, we successfully generated the signature matrix by executing the “Create Signature Matrix” module [16]. The resulting signature matrix was then applied to perform CIBERSORTx-based deconvolution analysis on the bulk RNA-seq data, with a statistical

significance threshold set at $P < 0.05$.

To visually represent the proportional distribution of distinct cell populations, we generated bar plots using the R packages “ggplot2” and “pheatmap” for comprehensive data visualization.

Metabolic pathway analysis

Gene set variation analysis (GSVA) is a nonparametric, unsupervised computational approach designed to assess gene set enrichment patterns in transcriptomic data derived from both microarray and RNA-seq platforms [18]. In this study, the GSVA R package was used to systematically evaluate the activity profiles of 81 hallmark metabolic pathways, thereby identifying pregnancy-associated metabolic pathways. Data visualization was performed using the R packages “ggplot2” and “pheatmap” to generate interpretable graphical representations.

Untargeted metabolomic analysis

Metabolomic profiling of the gastrocnemius muscle samples was performed via a Vanquish UHPLC system coupled with an Orbitrap Q Exactive HF-X mass spectrometer (LC-MS). Differentially abundant metabolites were identified through orthogonal partial least squares-discriminant analysis (OPLS-DA) with the threshold criteria of variable importance in projection (VIP) ≥ 1 and a P value < 0.05 . All the data were processed using the Omicsmart cloud platform (<https://www.omicsmart.com>). No multiple testing correction (such as Benjamini-Hochberg FDR correction) was applied owing to the exploratory nature of this study, which prioritized the sensitivity for detecting potential metabolic alterations.

Measurement of metabolite levels

The levels of metabolites in the gastrocnemius muscle were determined using the following commercial assay kits: the Succinic Acid Colorimetric Assay kit (Elabscience, Wuhan, China), the α -Ketoglutarate Fluorometric Assay kit (Elabscience), the Citric Acid Colorimetric Assay kit (Elabscience), and the Malic Acid Colorimetric Assay kit (Elabscience).

Cell culture and treatment

C2C12 cells (Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China) were maintained in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) for proliferation. To induce differentiation, the medium was replaced with differentiation medium consisting of DMEM containing 2% horse serum (HS) and 1% penicillin/streptomycin (P/S), and the cells were cultured for 3 days. During differentiation, the cells were treated with progesterone (#HY-N0437; MCE, Monmouth Junction, USA). The differentiated cells were subsequently exposed to insulin-containing DMEM for 3 h, after which the culture supernatant was collected. The glucose concentration in the supernatant was measured using a Glucose Assay kit (#F006-1-1; Njcbio, Nanjing, China). Glucose uptake was calculated as the difference between the initial glucose concentration in the basal medium and the glucose concentration in the supernatant after 3 h of insulin stimulation.

qRT-PCR analysis of core genes

Total RNA was extracted using TRIzol reagent (Takara, Dalian, China) and reverse-transcribed into cDNA. Gene expression was analyzed using TB Green Premix Ex Taq (Takara) with RPLP0 as the reference gene. Relative expression levels were calculated using the

$2^{-\Delta\Delta Ct}$ method. The primers were designed using Primer-BLAST (sequences are provided in [Supplementary Table S1](#)).

Statistical analysis

Data are expressed as the mean $\pm$ standard errors of the mean (SEM). Intergroup comparisons were performed via Student's t test, with P values < 0.05 considered statistically significant. All the statistical analyses and graphical representations were conducted via GraphPad Prism software (version 8.3.1).

Results

Metabolic disturbances in pregnant mice

Compared with non-pregnant control mice, pregnant mice presented significantly impaired glucose tolerance. Specifically, at 30 and 60 min following the glucose tolerance test (GTT), the blood glucose levels in pregnant mice were markedly higher than those in the control group ([Figure 1A](#)). This observation was further corroborated by the increased area under the curve (AUC) in pregnant mice ([Figure 1B](#)). Notably, no significant difference in fasting blood glucose levels (0-min timepoint) was detected between the two groups.

Consistent with glucose intolerance, insulin tolerance tests (ITTs) revealed marked insulin resistance in pregnant mice. Specifically, at 30, 60, and 90 min during the ITT, the blood glucose levels in pregnant mice were significantly greater than those in the control group ([Figure 1C](#)), with significant AUC elevation in pregnant mice ([Figure 1D](#)).

Lipid profiling revealed pregnancy-associated dyslipidemia characterized by elevated serum triglyceride (TG) levels ([Figure 1E](#)) along with reduced high-density lipoprotein cholesterol (HDL-C) levels ([Figure 1F](#)). The fiber morphology and organizational features showed that the skeletal muscle cross-sectional area (CSA) was bigger in Non-Preg group than that in Preg group ([Figure 1G, H](#)).

Pregnancy induces significant transcriptomic remodelling in skeletal muscle

RNA sequencing of skeletal muscle from Non-Preg and Preg mice generated high-quality data, with 39.56 to 53.64 million clean reads per sample ([Supplementary Table S2](#)). Principal component analysis (PCA) revealed clear separation between the groups, indicating that pregnancy status was the primary driver of transcriptional variation ([Figure 2A](#)). Comparative analysis revealed 1575 differentially expressed genes (DEGs, $|\log_2FC| > 1$, $P < 0.05$), with 902 upregulated genes and 673 downregulated genes ([Figure 2B,C](#)). Hierarchical clustering demonstrated robust pregnancy-associated transcriptional alterations ([Figure 2D](#)).

Pregnancy induces distinct pathways in skeletal muscle alterations

We identified key functional pathways and biological processes associated with the DEGs through KEGG and GO enrichment analyses. GO analysis revealed pregnancy-specific reprogramming of skeletal muscle. The upregulated genes were predominantly associated with metabolic adaptation and were enriched in carboxylic acid transmembrane transport, fat cell differentiation, the apical plasma membrane, membrane microdomains, membrane rafts, and L-amino/carboxylic/organic acid transmembrane transporter activity ([Figure 3A](#)). These findings suggest heightened

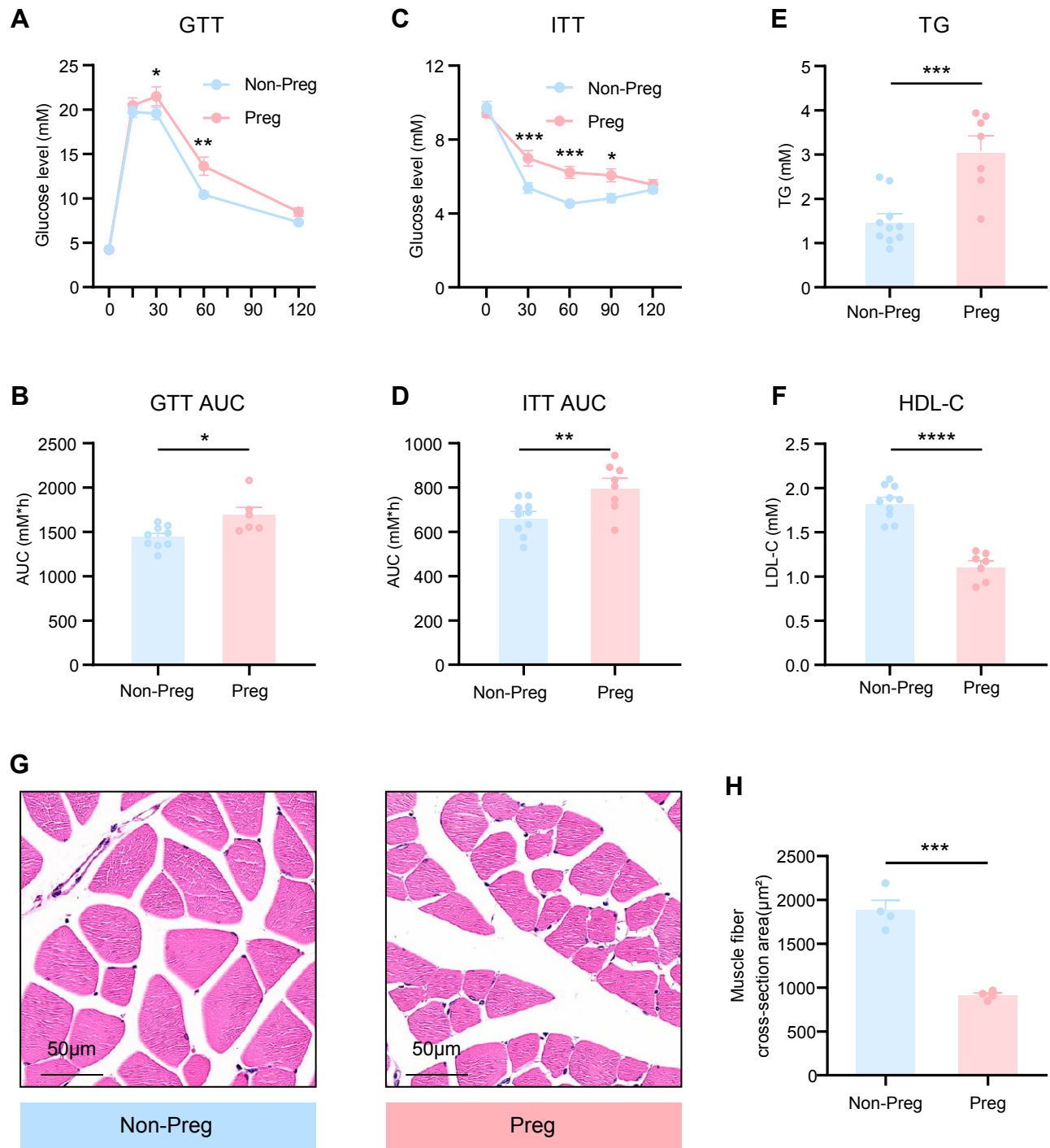


Figure 1. Pregnant mice exhibited metabolic disturbances (A) Glucose tolerance test (GTT) curves in Non-Preg and Preg groups. (B) Area under the curve (AUC) analysis of GTT results. (C) Insulin tolerance test (ITT) curves in Non-Preg and Preg groups. (D) AUC analysis of ITT results. (E) Triglyceride (TG) levels in Non-Preg and Preg groups. (F) High-density lipoprotein cholesterol (HDL-C) levels in Non-Preg and Preg groups. (G) Representative hematoxylin and eosin (H&E) stained sections of skeletal muscle tissue, illustrating general histoarchitecture. Images were acquired at 20 $\times$ magnification to visualize fiber morphology and organizational features. (H) Skeletal muscle cross-sectional area (CSA) was quantified from H&E stained sections ($n = 4$ per group). Scale bar: 50 μ m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

metabolic demands and enhanced intercellular interactions in skeletal muscle during pregnancy.

Conversely, downregulated genes reflected structural reorganization and metabolic reprioritization, with marked depletion in

sarcomeres, myofibrils, contractile muscle fibres, I bands, T-tubules, muscle cell development and muscle cell differentiation (Figure 3B). These results indicate that myofiber remodelling occurs in skeletal muscle during pregnancy. The observed reductions in

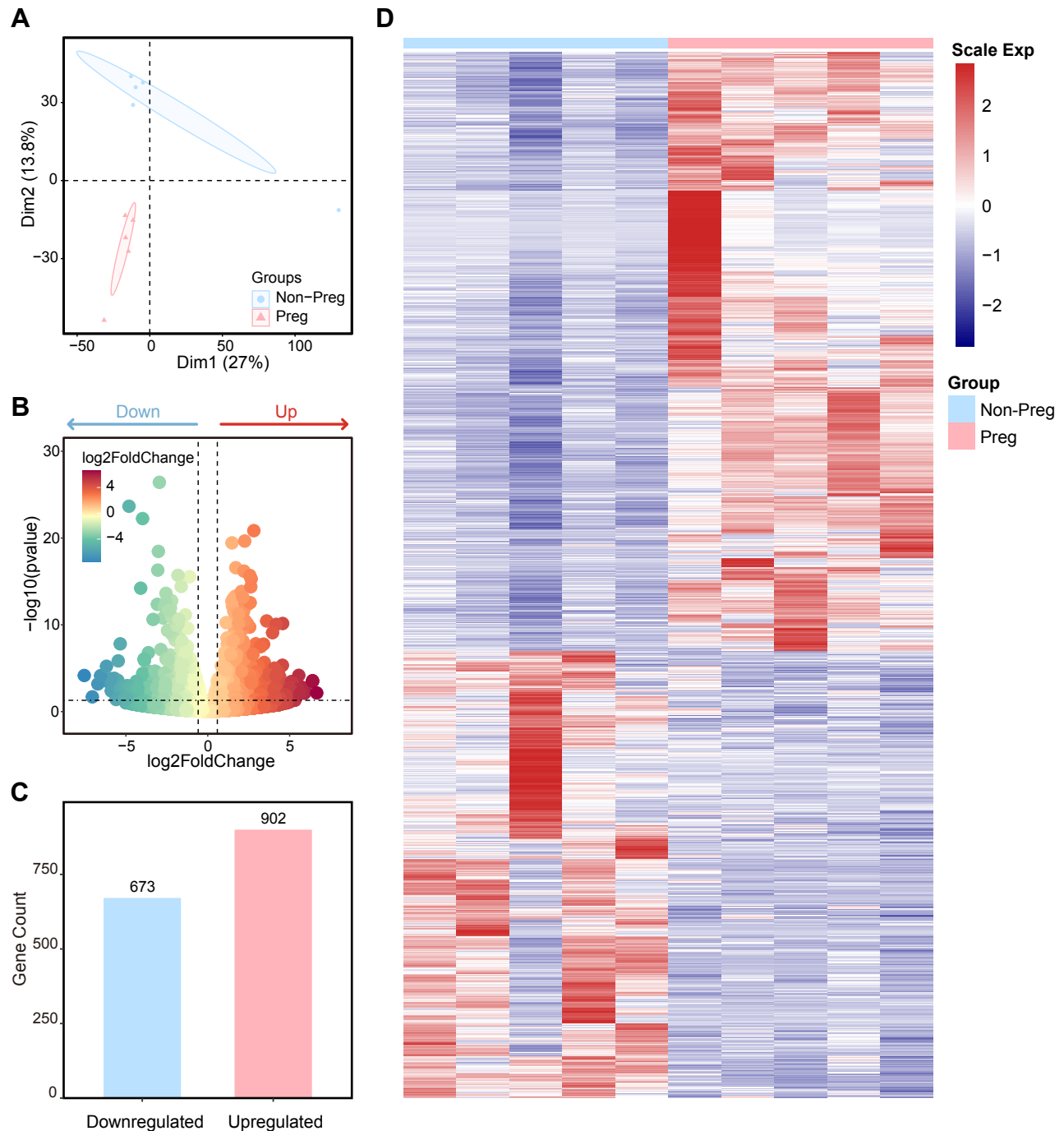


Figure 2. Pregnancy's effect on skeletal muscle transcriptome in pregnant mice (A) PCA plot demonstrating clear separation between Non-Preg and Preg groups based on global gene expression patterns. (B) Volcano plot visualizing DEGs between Non-Preg and Preg groups. (C) Bar plot showing the number of significantly DEGs between Non-Preg and Preg groups. (D) Heatmap displaying expression patterns of pregnancy-regulated genes, with samples clustered by similarity in gene expression profiles.

molecular functions—primarily involving NADPH binding, DNA-dependent protein kinase activity, and AMP-activated protein kinase activity—suggest the presence of underlying energy metabolism dysfunction. These coordinated changes demonstrate a physiological shift toward enhanced nutrient transport capacity at the expense of myofiber integrity and oxidative metabolism, which is consistent with the metabolic demands of

pregnancy.

The KEGG pathway analysis revealed significant enrichment of DEGs in signal transduction-related pathways (148 genes), with distinct regulatory patterns between upregulated and downregulated genes (Figure 4A). The downregulated pathways included the PI3K-AKT signaling pathway and the TGF-beta signaling pathway (Figure 4B), both of which are known to maintain insulin sensitivity

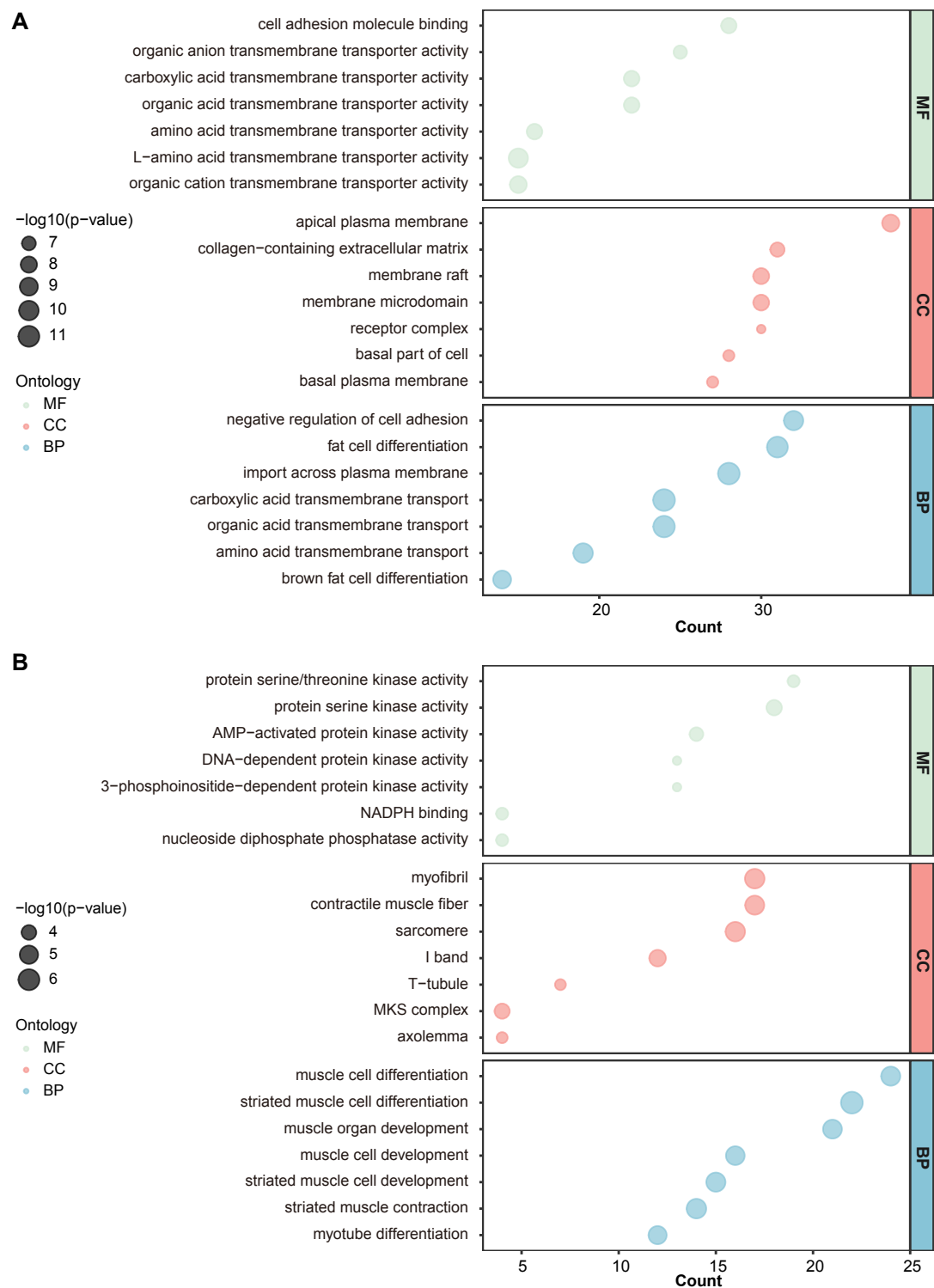
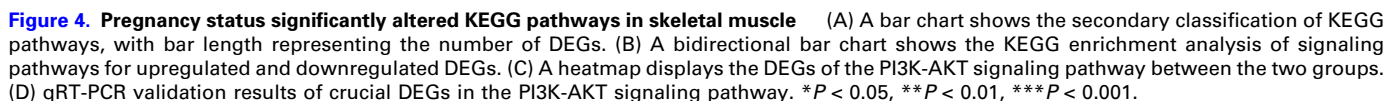


Figure 3. Pregnancy status significantly altered GO biological pathways in skeletal muscle (A) GO terms significantly enriched in genes upregulated in Preg vs Non-Preg groups. (B) GO terms significantly enriched in genes downregulated in Preg vs Non-Preg groups.

[19,20]. Additionally, the HIF1 signaling pathway and MAPK signaling pathway were significantly upregulated in the Preg group, and their activation is typically associated with the promotion of insulin resistance [21,22].

Heatmap visualization (Figure 4C) and RT-qPCR confirmed the

consistent downregulation of key PI3K-AKT pathway components, including *Angpt1*, *Ccne2*, *Egf*, *Itgb6*, *Ntf5*, and *Itga4* (Figure 4D). These results suggest that pregnancy drives skeletal muscle toward an insulin-resistant state by simultaneously suppressing insulin-sensitizing pathways (PI3K-AKT and TGF- β) while activating stress-



Single-cell deconvolution reveals pregnancy-associated cellular remodelling in skeletal muscle

To investigate the changes in the cellular composition of maternal

skeletal muscle during pregnancy, we employed CIBERSORTx to perform computational deconvolution of bulk RNA-seq data using reference single-cell dataset (Figure 5A,B). We identified two key aspects of pregnancy-associated skeletal muscle remodelling, enabling the quantification of the cellular abundance of each sample (Figure 5B). First, our analysis revealed a significant reduction in the abundance of muscle stem cell (MuSC) populations in pregnant skeletal muscle (Figure 5C), along with qPCR-confirmed downregulation of the MuSC marker gene *Pax7* and

myogenic regulatory factors (*Myod1*, *Myog*, *Myf5*, and *Mef2c*) (Figure 5D). However, the enhanced regenerative capacity was not attributed to an increase in satellite cell quantity but rather to the activation of Notch signaling [23]. Further GSVA revealed significantly higher Notch signaling scores in the Preg group than in the control group (Figure 5E). These findings suggest that MuSCs from pregnant mice possess increased regenerative potential.

Concurrently, we observed increased proportions of endothelial cells in pregnant skeletal muscle (Figure 5F). Gene set enrichment

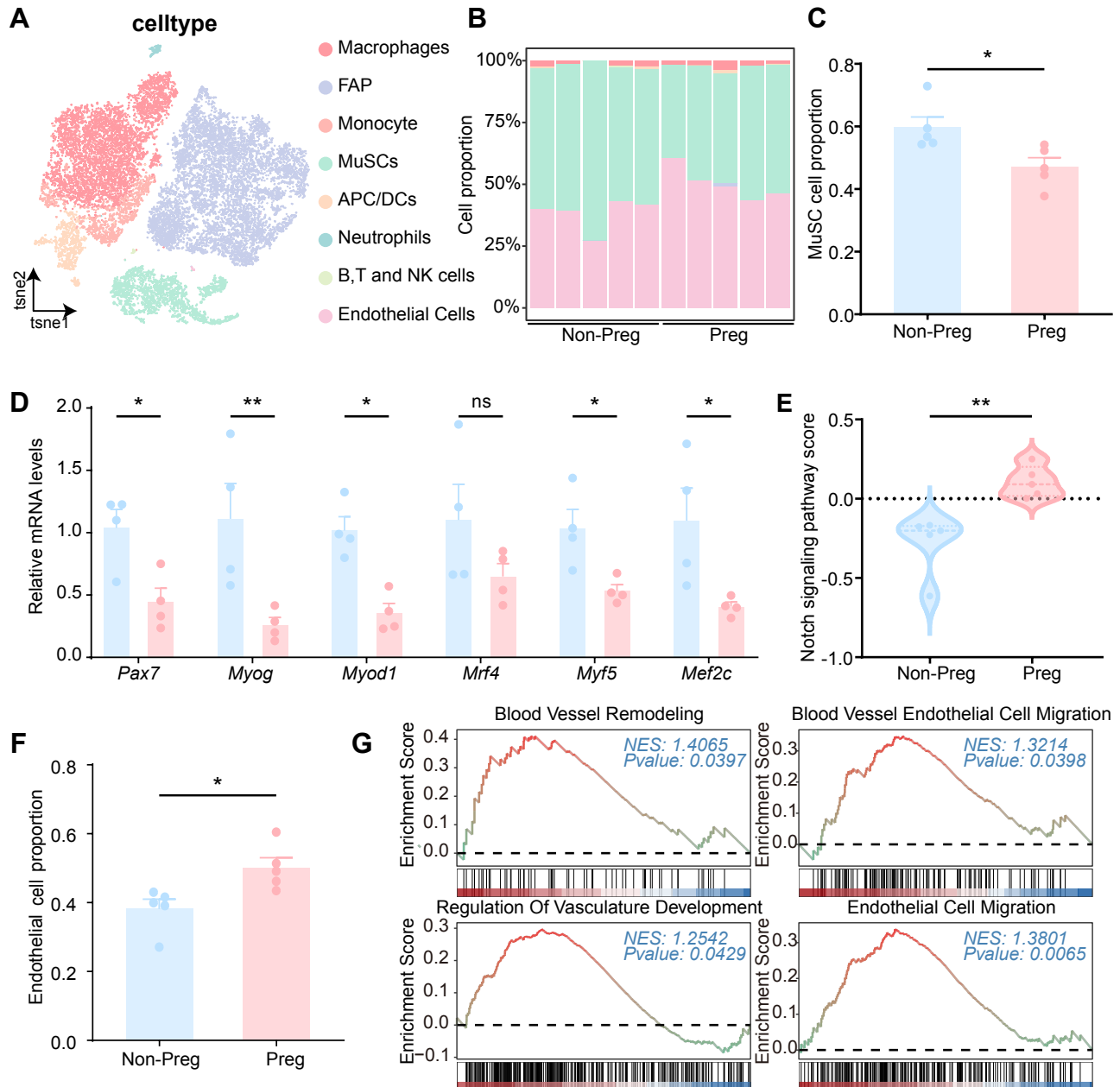


Figure 5. Single-cell deconvolution reveals pregnancy-associated cellular remodelling in skeletal muscle (A) UMAP visualization of all cells color-coded by annotated cell types in muscle scRNA-seq data. (B) Stacked bar plot displaying the cellular composition distribution across bulk RNA-seq samples. (C) Bar plot quantifying the proportional abundance of MuSCs. (D) Bar plot demonstrating expression levels of MuSC marker genes in whole skeletal muscle tissue, as confirmed by qPCR. (E) Violin plots depicting Notch signaling pathway scores derived from GSVA analysis. (F) Bar plot representing the proportional abundance of endothelial cells. (G) Gene set enrichment analysis (GSEA) revealing endothelial cell-associated pathways. * $P < 0.05$, ** $P < 0.01$. ns, not significant.

analysis (GSEA) demonstrated significant enrichment of vascular remodelling pathways, including blood vessel remodelling, blood vessel endothelial cell migration, and regulation of vasculature development (Figure 5G). These coordinated changes reflect an adaptive strategy wherein skeletal muscle prioritizes vascular support for fetal-maternal metabolic demands while temporarily suppressing energy-intensive myogenic processes, potentially

through Notch-mediated modulation of remaining MuSC activity.

Metabolic pathway remodelling in skeletal muscle during pregnancy

Gene set variation analysis (GSVA) of 81 metabolic pathways revealed 11 significantly altered metabolic pathways when comparing the Non-Preg and Preg groups (Figure 6A,B). Notably, both the

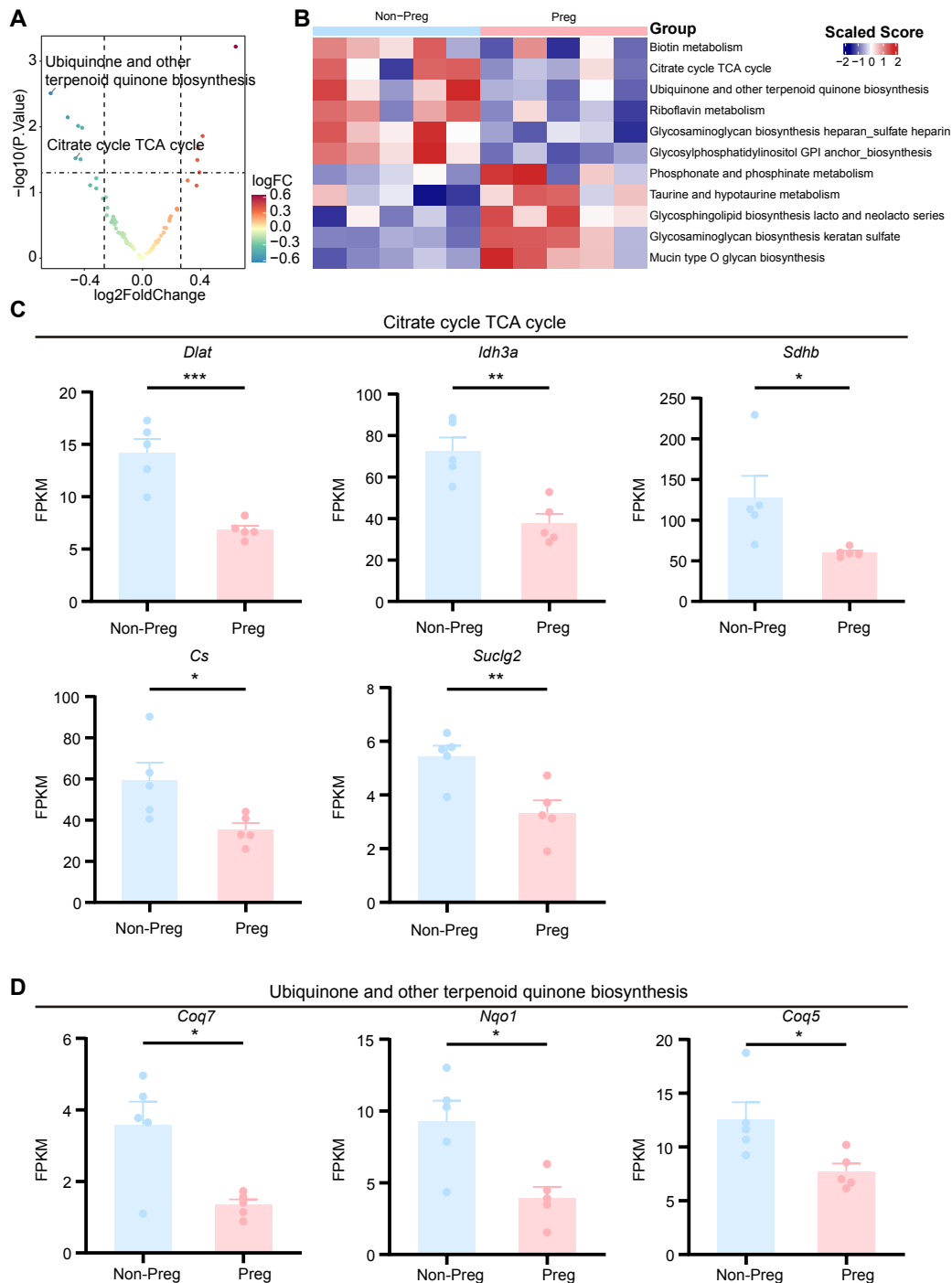


Figure 6. Metabolic pathway analysis reveals pregnancy-induced alterations in metabolic profiles (A) Volcano plot highlighting significantly altered metabolic pathways between Non-Preg and Preg groups. (B) Heatmap showing hierarchical clustering of significantly changed metabolic pathways induced by pregnancy. (C) Bar plot comparing expression levels of TCA cycle-related genes between Non-Preg and Preg groups. (D) Bar plot comparing expression levels of quinone biosynthesis-related genes between non-Preg and Preg groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

citrate cycle-TCA cycle and the ubiquinone/terpenoid-quinone biosynthesis pathways were markedly downregulated in the Preg group. This was accompanied by coordinated suppression of key metabolic enzymes, including TCA cycle components (*Dlat*, *Idh3a*, *Sdhb*, *Cs*, and *Suc1g2*) and ubiquinone biosynthesis factors (*Coq7*, *Nqo1*, and *Coq5*) (Figure 6C,D). These findings suggest a physiological adaptation wherein skeletal muscle reduces oxidative metabolism during pregnancy, potentially serving to redirect nutrients toward fetal development, limit reactive oxygen species production, and optimize energy allocation in response to gestational metabolic demands. The observed metabolic remodelling provides a molecular basis for understanding the trade-off between maternal tissue maintenance and fetal growth requirements during pregnancy.

Pregnancy induces profound metabolic remodelling in skeletal muscle

LC-MS/MS-based metabolomics identified a total of 2,357 MS2-validated metabolites that clearly distinguished pregnant skeletal muscle from nonpregnant skeletal muscle. Multivariate statistical analyses, including PCA, PLS-DA, and OPLS-DA, were applied to assess metabolic variations among the groups, ensuring data quality and screening for differentially abundant metabolites (Figure 7).

PCA of the Non-Preg and Preg groups revealed clear separation, indicating significant metabolic disturbances in the Preg group (Figure 7A). This discrimination was enhanced by supervised PLS-DA modelling, with OPLS-DA confirming significant metabolic perturbations after accounting for biological variability, and the significant clustering confirmed the reliability of the metabolic variations (Figure 7B,C). The consistent clustering patterns across all multivariate approaches validated pregnancy-specific metabolic reprogramming in skeletal muscle.

We subsequently applied selection criteria of VIP > 1 from the OPLS-DA model combined with a significance threshold of $P < 0.05$ to identify differentially expressed metabolites. Comparative analysis revealed 95 significantly dysregulated metabolites (16 upregulated and 79 downregulated) in the Preg group (Figure 7D). To elucidate the biological significance of these metabolic alterations, KEGG pathway enrichment analysis revealed predominant changes in cysteine and methionine metabolism, sphingolipid metabolism, and arginine and proline metabolism, among others (Figure 7E). These findings suggest that skeletal muscle undergoes substantial metabolic remodelling during pregnancy. Notably, multiple TCA cycle intermediates, including succinates, hydroxypyruvic acid, itaconic acid, danthron, linalyl acetate, and p-cresol, exhibited coordinated downregulation (Figure 7F). These metabolic shifts suggest that physiological adaptations prioritize redox balance in maternal tissues through modified amino acid and lipid metabolism. The comprehensive metabolic changes validated by MS/MS fragmentation patterns and multivariate model statistics provide a systems-level view of skeletal muscle adaptation to pregnancy. We also measured the levels of succinic acid, α -ketoglutarate, citric acid, and malic acid in the gastrocnemius muscle. The results revealed that the levels of these metabolites were decreased in the gastrocnemius muscle of pregnant mice (Supplementary Figure S1).

Progesterone recapitulates gestational transcriptional adaptations and metabolism *in vitro*

Pregnancy is accompanied by a series of maternal hormonal

alterations, including changes in estrogen, progesterone, human chorionic gonadotropin (HCG), and human placental lactogen (HPL). To investigate whether pregnancy-related hormones contribute to adaptive metabolic changes in skeletal muscle during gestation, we specifically examined the role of progesterone, which has previously been reported to induce insulin resistance at the physiological pregnancy level. We stimulated C2C12 myotubes with progesterone at concentrations mimicking physiological pregnancy conditions. Our results demonstrated that progesterone significantly reduced glucose uptake in C2C12 cells (Figure 8A). Furthermore, we assessed whether the adaptive changes observed in gestational muscle were recapitulated in progesterone-treated C2C12 cells. We found that the expression of myogenic regulatory genes (such as *Pax7* and *Mef2c*; Figure 8B) as well as key genes involved in glucose metabolic pathways (including *Ccne2*, *Egf*, *Itg6b*, *Ntf5*, and *Itga4*; Figure 8C) was significantly downregulated.

High-fat diet exacerbates transcriptional alterations and metabolic dysregulation in skeletal muscle during pregnancy

To investigate the impact of pregnancy-induced adaptive changes under pathological conditions, we established a HFD mouse model during gestation. After two weeks of pre-pregnancy HFD feeding followed by continued HFD exposure throughout pregnancy, the HFD-fed mice presented significant glucose metabolic abnormalities. Specifically, during the GTT, blood glucose levels at 30, 60, and 90 min were significantly higher in the HFD group than in the NCD (control) group (Figure 9A). The AUC for the GTT was also elevated in the HFD group (Figure 9B). In the ITT, blood glucose levels at 30 min were higher in the HFD group than in the NCD control group (Figure 9C), and the AUC for the ITT was also increased in the HFD group (Figure 9D).

Our preliminary data from this model indicate that the expression of key genes involved in gestational adaptive changes—particularly those related to glucose metabolism (such as *Angpt1*, *Itgb6*, *Ntf5*, and *Itga4*; Figure 9E) and myogenesis (including *Mrf4* and *Mef2c*; Figure 9F)—is further dysregulated in HFD-fed mice compared with normal pregnant controls. Furthermore, we observed a notable reduction in the levels of TCA cycle metabolites, including citrate, succinate, α -ketoglutarate, and malate, in the skeletal muscle of HFD-fed mice (Figure 9G).

Discussion

Pregnancy induces profound metabolic remodelling in skeletal muscle, representing a physiological adaptation to meet the growing nutritional demands of the fetus [24]. Our integrated multi-omics analysis revealed that this adaptation is characterized by coordinated changes at the transcriptional, cellular, and metabolic levels. The observed maternal insulin resistance, while traditionally viewed as pathological, likely serves as an essential metabolic switch to prioritize glucose allocation for fetal development. Current evidence indicates that pregnancy-induced insulin resistance exhibits tissue-specific patterns across metabolic organs [8]. While both adipose tissue and skeletal muscle display impaired insulin signaling, hepatic insulin sensitivity remains relatively unaffected [8]. Notably, previous research on maternal metabolic adaptation has focused predominantly on adipose tissue, with insufficient attention given to skeletal muscle [25–27]. Given the central role of skeletal muscle in systemic glucose metabolism and

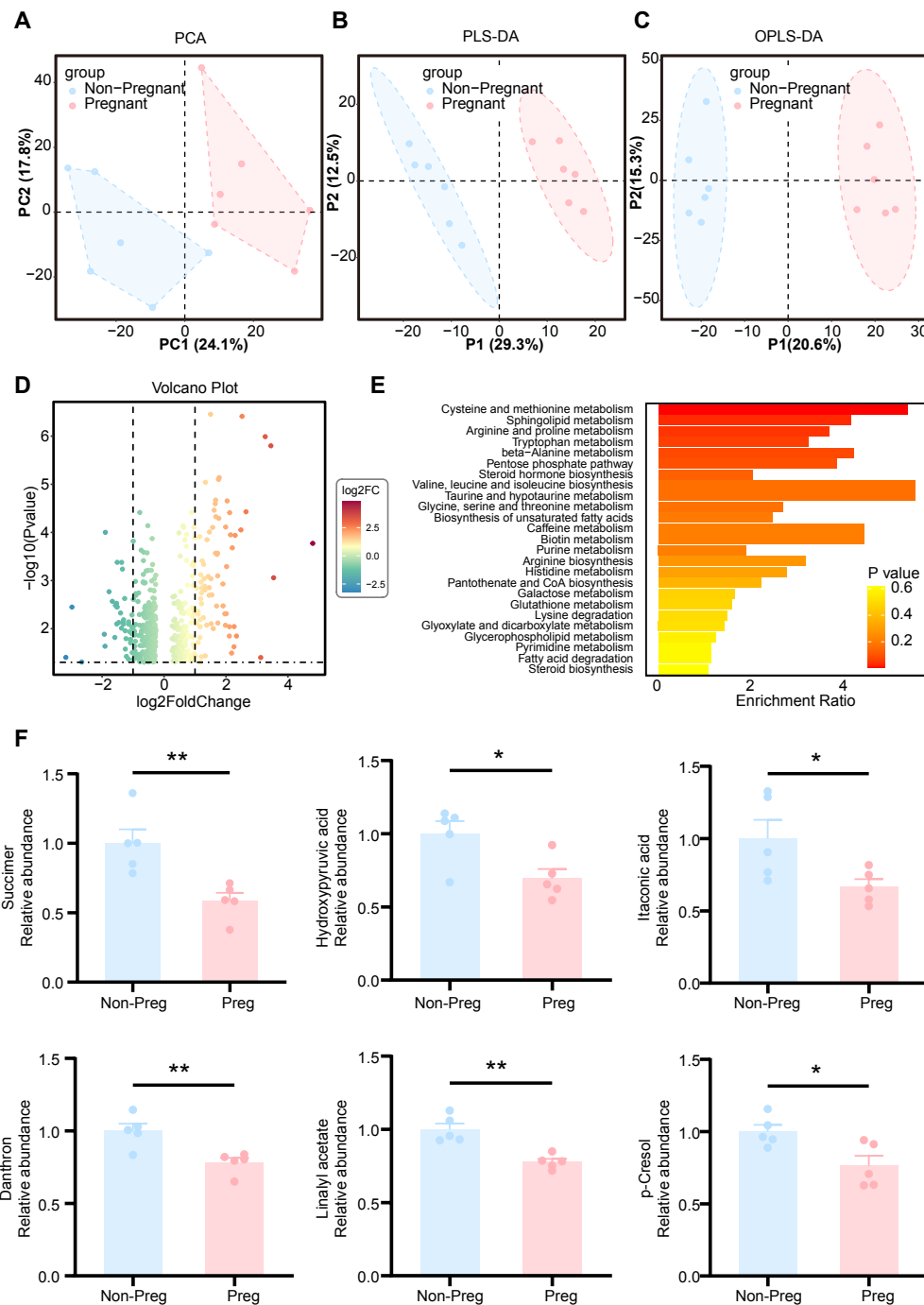


Figure 7. Pregnancy's effect on skeletal muscle metabolome in pregnant mice (A) PCA plot showing sample clustering and group separation between Non-Preg and Preg groups. (B) PLS-DA plot demonstrating intragroup sample correlations and intergroup discrimination. (C) OPLS-DA plot highlighting metabolic variability between Non-Preg and Preg groups. (D) Volcano plot visualizing significantly altered metabolites between Non-Preg and Preg groups. (E) Bar plot showing top enriched metabolic pathways, with bar length representing enrichment ratio and color indicating statistical significance. (F) Bar plot comparing TCA cycle and quinone biosynthesis metabolite levels between Non-Preg and Preg groups. * $P < 0.05$, ** $P < 0.01$.

its strong association with insulin resistance [28], a systematic elucidation of its molecular metabolic features during pregnancy is of critical scientific importance.

Transcriptomic profiling revealed 1575 DEGs in skeletal muscle between the pregnant and non-pregnant groups, highlighting enhanced nutrient transport capacity through the upregulation of

transmembrane transporters, alongside structural reorganization via the downregulation of contractile apparatus components. This molecular signature aligns with a pregnancy-induced fiber-type transition from slow-twitch type I to fast-twitch type IIa fibers in murine skeletal muscle [29]. Type I fibers, characterized by their oxidative metabolic profile dependent on fatty acid and glucose

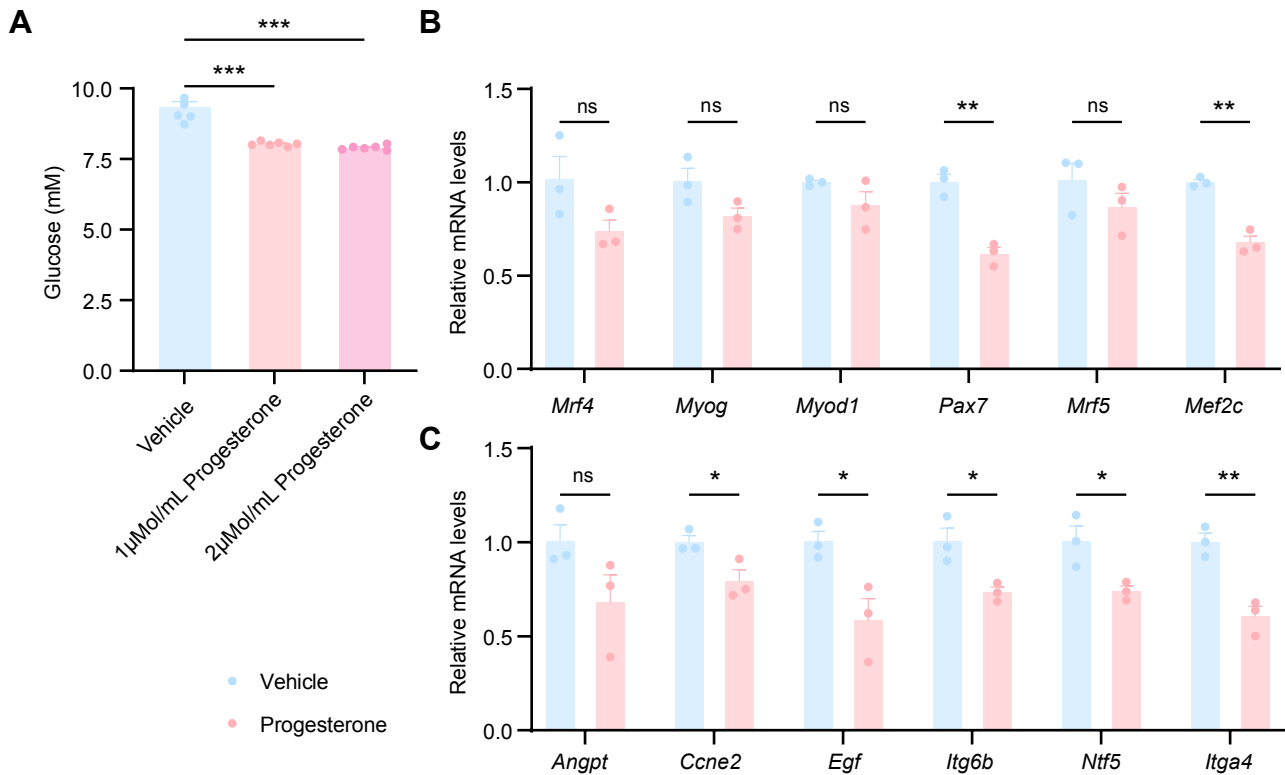


Figure 8. Progesterone recapitulates gestation's transcriptional and metabolic adaptations *in vitro* (A) Bar plot demonstrating glucose consumption in the culture supernatant. (B) Bar plot demonstrating the expression levels of MuSC marker genes, as confirmed by qPCR. (C) qRT-PCR validation results of crucial DEGs in the PI3K-AKT signaling pathway. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, not significant.

oxidation, typically exhibit high mitochondrial density and extensive capillary networks to support sustained aerobic activity [30]. In contrast, type IIa fibers demonstrate enhanced contractile velocity and power generation capabilities while maintaining moderate oxidative capacity, representing a hybrid phenotype that combines glycolytic and oxidative metabolic features [31]. This adaptive shift in fiber-type composition likely serves as a physiological strategy to optimize maternal musculoskeletal function during gestation, enabling both rapid force production when needed and metabolic flexibility to accommodate the dynamic energy demands of pregnancy.

Our transcriptomic analysis revealed significant enrichment of DEGs in signal transduction pathways. Notably, we observed downregulation of the PI3K-AKT and TGF- β signaling pathways during pregnancy, whereas the MAPK and HIF signaling pathways were upregulated. The key molecules implicated in impaired insulin signaling include IRS, PI3K, Akt, and GSK-3 β [32]. The downregulation of the PI3K-AKT pathway likely reflects an adaptive decrease in skeletal muscle insulin sensitivity during gestation. Wang *et al.* [33] demonstrated that TGF- β specifically inhibits intramuscular fat deposition, suggesting that the attenuation of TGF- β signaling in maternal muscle may contribute to gestational lipid accumulation. Inflammatory mediators can activate the MAPK pathway, leading to JNK-mediated phosphorylation of serine and threonine residues on IRS1, which in turn suppresses tyrosine phosphorylation of IRS1. This impairs PI3K/Akt signaling and GLUT4 translocation, ultimately resulting in reduced insulin utilization in peripheral tissues and insulin resistance (IR) [34].

Furthermore, transgenic mice overexpressing HIF1 α exhibited exacerbated obesity and insulin resistance accompanied by increased inflammation and fibrosis [35,36], highlighting the potential role of HIF signaling in metabolic dysregulation during pregnancy. These coordinated alterations in core signaling networks demonstrate the complex endocrine-metabolic reprogramming during pregnancy, which achieves a balance between fetal growth and maternal homeostasis through precise regulation of these evolutionarily conserved pathways.

Single-cell deconvolution further revealed a significant reduction in MuSC populations coupled with an increase in endothelial cell populations in pregnant groups, suggesting a trade-off between myogenic potential and nutrient delivery efficiency. Given the pivotal role of MuSCs in myocyte regeneration, we validated these findings via the quantification of the expression of the MuSC marker gene *Pax7* [37] and myogenic regulatory factors [38] (*Myod1*, *Myog*, *Myf5*, and *Mef2c*) via qPCR. However, previous studies have demonstrated that pregnant mice exhibit accelerated muscle healing following injury [23,39], which contradicts our conclusion. Notably, research has indicated that this enhanced regenerative capacity is not attributable to changes in MuSC quantity but rather to activation of Notch signaling in MuSCs mediated by pregnancy-associated serum factors [39]. We therefore hypothesize that although the absolute number of MuSCs is reduced in pregnant mice, their differentiation potential may be greater than that of their nonpregnant counterparts, thereby conferring superior regenerative capacity in response to acute injury. Furthermore, the observed expansion of endothelial cells likely reflects increased nutrient

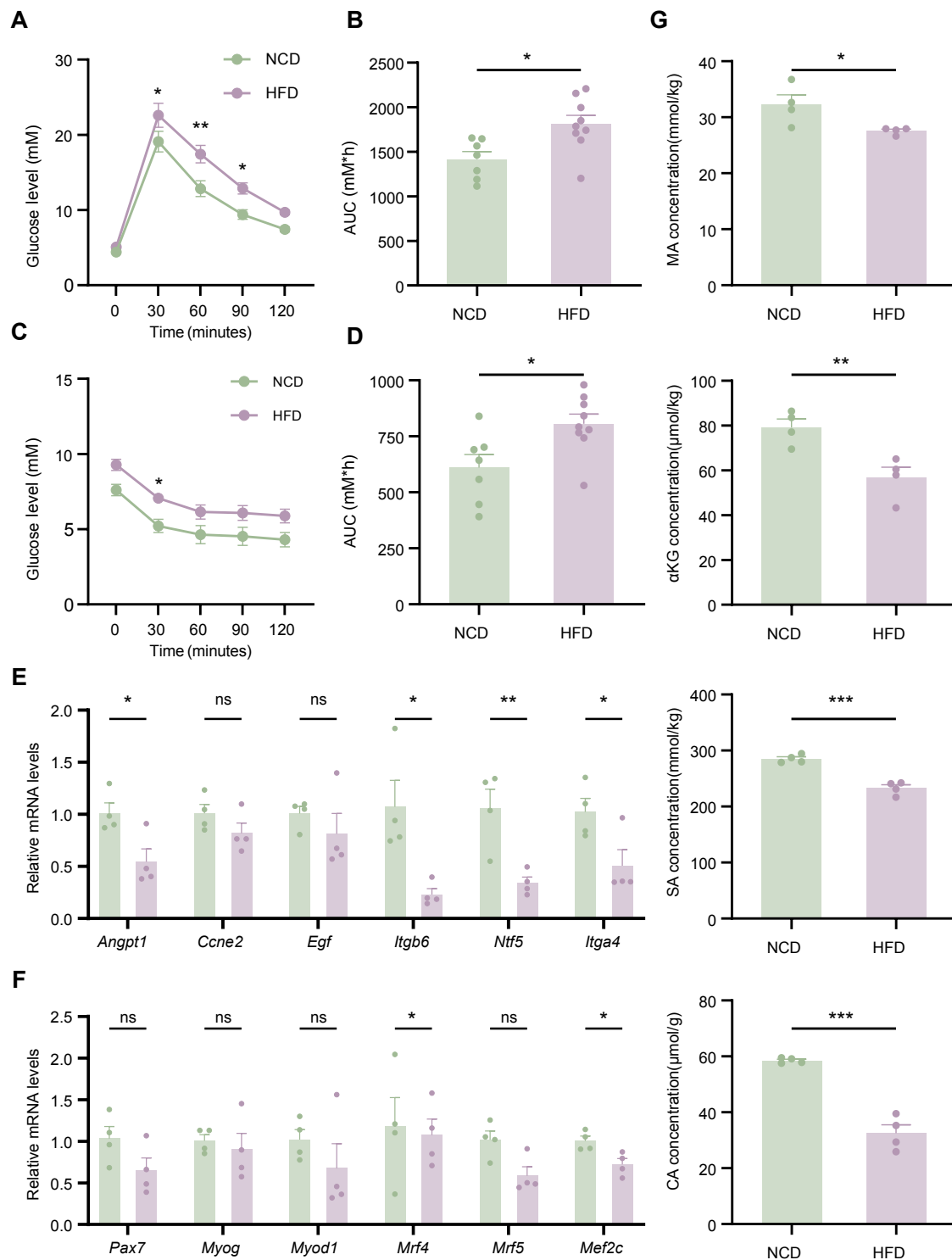


Figure 9. High-fat diet exacerbates transcriptional alterations and metabolic dysregulation in skeletal muscle during pregnancy (A) Glucose tolerance test (GTT) curves of the NCD and HFD groups. (B) Area under the curve (AUC) analysis of the GTT results. (C) Insulin tolerance test (ITT) curves of the NCD and HFD groups. (D) AUC analysis of the ITT results. (E) qRT-PCR validation results of crucial DEGs in the PI3K-AKT signaling pathway. (F) Bar plot demonstrating the expression levels of MuSC marker genes in whole skeletal muscle tissue, as confirmed by qPCR. (G) Abundance of typical differentially abundant metabolites in the NCD and HFD groups ($n = 4$ per group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, not significant.

delivery demands during gestation, which is consistent with the upregulation of genes involved in amino acid transmembrane

transport activity. Collectively, these cellular and molecular adaptations represent key features of maternal metabolic repro-

gramming during pregnancy.

Metabolic pathway analysis revealed significant metabolic reprogramming characteristics in skeletal muscle during pregnancy, with particularly prominent downregulation of the tricarboxylic acid (TCA) cycle and ubiquinone biosynthesis pathways, two crucial pathways intimately associated with mitochondrial oxidative phosphorylation function. This finding corroborates the results of the GO enrichment analysis showing downregulated expression of NADPH binding-related genes, collectively suggesting a potential reduction in the efficiency of mitochondrial energy metabolism in gestational skeletal muscle. Previous studies have demonstrated a pregnancy-associated shift in maternal skeletal muscle fiber composition from type I to type IIa [30,40], indicating a metabolic transition from predominantly oxidative phosphorylation to glycolytic metabolism [41], resulting in reduced mitochondrial energy production. This phenotypic conversion may underlie the observed downregulation of the TCA cycle and related pathways in gestational skeletal muscle. Further metabolomic analysis not only revealed dynamic changes in overall metabolites in pregnant muscle tissue but also, more specifically, verified alterations in metabolite concentrations associated with the TCA cycle and ubiquinone biosynthesis pathways. These results provide compelling evidence at the metabolic flux level for an adaptive shift from oxidative metabolism toward glycolytic metabolism in skeletal muscle during pregnancy.

During pregnancy, significant changes occur in the levels of various hormones, including estrogen, progesterone, human chorionic gonadotropin (HCG), and placental lactogen. Previous studies have reported that progesterone at physiological pregnancy levels can impair insulin sensitivity and promote the development of insulin resistance. Our findings demonstrate that progesterone induces transcriptional and metabolic alterations in skeletal muscle cells that resemble the adaptive remodelling observed in gestational skeletal muscle. Moreover, a high-fat diet was shown to exacerbate these adaptive changes. This finding suggests that while such adaptations under physiological conditions facilitate preferential nutrient allocation to the fetus, the superimposition of adverse factors such as obesity may disrupt metabolic homeostasis, thereby increasing the risk of pregnancy-related complications, such as gestational diabetes mellitus.

Although Lontay *et al.* [41] previously reported microarray data from the skeletal muscle of *Smtnl1*^{+/+} and *Smtnl1*^{-/-} non-pregnant and 14-day pregnant animals, their study focused primarily on investigating the effects of SMTNL1 on gestational skeletal muscle rather than the intrinsic impact of pregnancy itself. Although Yu *et al.* [42] characterized the systemic metabolome during pregnancy in cynomolgus monkeys, they did not systematically examine pregnancy-associated adaptations in skeletal muscle. Therefore, our study represents the first integrated multi-omics analysis to delineate both the molecular and metabolic signatures underlying skeletal muscle adaptation during pregnancy, providing novel insights into pregnancy-related metabolic regulation. These findings not only advance our understanding of gestational physiology but also identify potential therapeutic targets for pregnancy-associated metabolic disorders. Future investigations should further elucidate the spatiotemporal relationship between these adaptive changes and the development of gestational insulin resistance, as well as their functional roles in postpartum metabolic recovery.

This study provides the first integrated multi-omics characteriza-

tion of gestational skeletal muscle adaptations in murine models. We observed abnormal metabolic parameters, including impaired glucose tolerance, insulin resistance, and dysregulated blood lipid levels, in pregnant mice. Further analysis revealed that the skeletal muscle of pregnant mice presented downregulation of genes involved in myofiber assembly, reduced myocyte numbers, and increased endothelial cell content. Additionally, we investigated alterations in skeletal muscle metabolism and reported decreased expression of genes and metabolites related to the TCA cycle and ubiquinone synthesis. Collectively, our data suggest that muscle tissue may play a role in metabolic regulation during human pregnancy.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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